

**THIS PAGE IS INSERTED BY OIPE SCANNING
AND IS NOT PART OF THE OFFICIAL RECORD**

Best Available Images

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

BLACK BORDERS

TEXT CUT OFF AT TOP, BOTTOM OR SIDES

FADED TEXT

BLURRY OR ILLEGIBLE TEXT

SKEWED/SLANTED IMAGES

COLORED PHOTOS HAVE BEEN RENDERED INTO BLACK AND WHITE

VERY DARK BLACK AND WHITE PHOTOS

UNDECIPHERABLE GRAY SCALE DOCUMENTS

**IMAGES ARE THE BEST AVAILABLE
COPY. AS RESCANNING *WILL NOT*
CORRECT IMAGES, PLEASE DO NOT
REPORT THE IMAGES TO THE
PROBLEM IMAGE BOX.**

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 December 2000 (28.12.2000)

PCT

(10) International Publication Number
WO 00/78738 A1

(51) International Patent Classification⁷: C07D 251/70, A61K 31/53, A61P 35/00, 35/02

(21) International Application Number: PCT/US00/05319

(22) International Filing Date: 29 February 2000 (29.02.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/338,679 23 June 1999 (23.06.1999) US

(71) Applicant: PARKER HUGHES INSTITUTE [US/US];
2665 Long Lake Road, St. Paul, MN 55113 (US).

(72) Inventors: UCKUN, Fatih, M.; 12590 Ethan Avenue North, White Bear Lake, MN 55110 (US). LIU, Xing-Ping; 1010 6th Street S.E., Minneapolis, MN 55414 (US). NARLA, Rama, K.; 1270 Larpenr Avenue, St. Paul, MN 55113 (US).

(74) Agent: DAIGNAULT, Ronald, A.; Merchant & Gould P.C., 3100 Norwest Center, 90 South Seventh Street, Minneapolis, MN 55402-4131 (US).

(81) Designated States (*national*): AE, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— With international search report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MELAMINE DERIVATIVES AS POTENT ANTI-CANCER AGENTS

(57) Abstract: Novel melamine derivatives having potent cytotoxic activity are described as anti-tumor agents and are particularly effective against leukemia and breast tumor cells.

WO 00/78738 A1

MELAMINE DERIVATIVES AS POTENT ANTI-CANCER AGENTS

Field of the Invention

5 This invention relates to melamine derivatives effective for treating tumor cells and particularly effective to induce apoptosis in leukemia and breast tumor cells.

Background of the Invention

10 Cancer is a major disease that continues as one of the leading causes of death at any age. In the United States alone, it is anticipated that more than a half a million Americans will die of cancer in 1999. Currently, radiotherapy and chemotherapy are two important methods used in the treatment of cancer.

15 Considerable efforts are underway to develop new chemotherapeutic agents for more potent and specific anti-cancer therapy, presenting effective and efficient cytotoxicity against tumor cells, with minimal interference with normal cell function. Accordingly, there is an urgent need for the development and analysis of novel, effective anti-cancer agents.

20

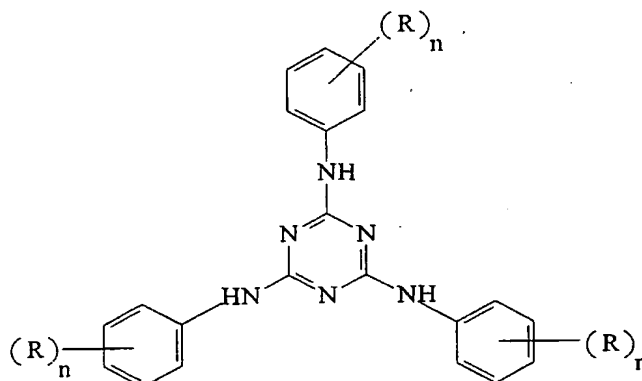
Summary of the Invention

 Novel derivatives of melamine have been found to be potent cytotoxic agents with potent activity against cancer cells. For example, certain melamine derivatives were found to exhibit potent cytotoxic activity, particularly against human breast cancer and leukemic cell lines, at micromolar concentrations.

25

 Accordingly, the present invention includes novel compounds and compositions as cytotoxic therapeutic agents, and particularly as anti-tumor agents. The present invention also includes methods for killing tumor cells and/or inhibiting the growth of tumors by administering to a subject an effective antitumor amount of a compound of the invention. Compositions of the invention contain an effective
30 cytotoxic or inhibitory amount of a melamine derivative.

The melamine compounds of the invention have following formula:



5 wherein each R is independently selected from the substituents described more fully below, and each n is independently in the range of 1 to 5, preferably 1-3.

Preferred compounds of the invention are those where each R independently comprises one or more halo or hydroxy group, such as those in
10 compounds shown in Table 2 below. Most preferred is 2, 4, 6-tri(3',5'-dichloro-4'-hydroxy phenyl) amino-1,3,5-triazine (WHI-P239).

Detailed Description of the Invention

The present invention includes novel melamine derivatives having
15 potent activity as cytotoxic agents. The compounds of the invention are useful agents in the treatment of cancer for example to inhibit tumor growth or to kill tumor cells, for example, leukemia and breast tumor cells.

Definitions

20 All scientific and technical terms used in this application have meanings commonly used in the art unless otherwise specified. As used in this application, the following words or phrases have the meanings specified.

As used herein, "alkyl", which includes the alkyl group of "alkoxy" substituents, includes both branched and straight-chain saturated aliphatic
25 hydrocarbon groups having the specified number of carbon atoms. As a preferred

embodiment, chains of 1 to 4 carbon atoms are included, for example methyl, ethyl, propyl, isopropyl, butyl, secondary butyl, t-butyl, and the like.

As used herein "halogen" or "halo" substituent includes fluoro, chloro, bromo, and iodo.

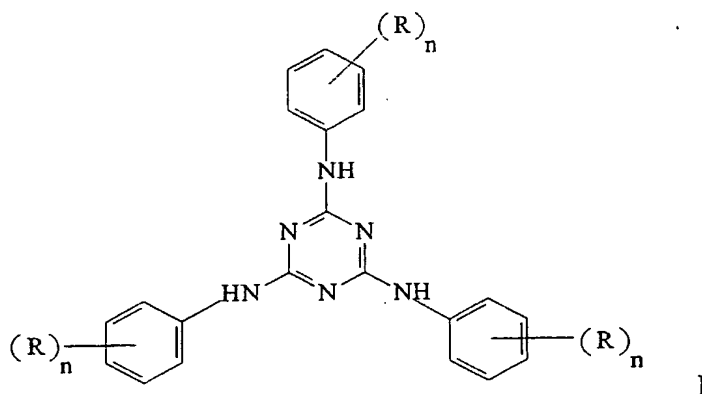
5 As used herein, "pharmaceutically acceptable salt thereof" includes an acid addition salt or a base salt.

As used herein, "pharmaceutically acceptable carrier" includes any material which, when combined with a compound of the invention, allows the compound to retain biological activity, such as the ability to induce apoptosis of
10 leukemia or breast tumor cells, and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsions, and various types of wetting agents. Compositions comprising such carriers are formulated by well known conventional methods (see, for example,
15 Remington's Pharmaceutical Sciences, Chapter 43, 14th Ed., Mack Publishing Co., Easton, PA).

"Treating" or "Treatment" in the context of this invention means the prevention or reduction of severity symptoms or effect of a pathological condition, including prolonging life expectancy. In the context of cancer therapy, treatment
20 includes prevention, of tumor growth, reduction of tumor size, enhanced tumor cell death, and increased apoptosis.

Compounds of the Invention

The novel melamine derivatives of the invention have the general structure represented by the following formula I:

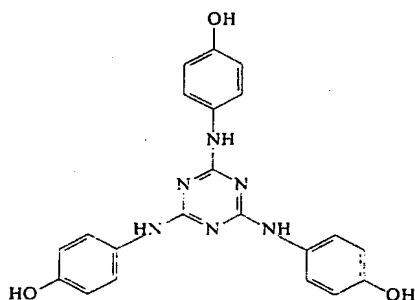


5 Where each n is independently an integer ranging from 1 to 5 (inclusive), and preferably is 1 to 3.

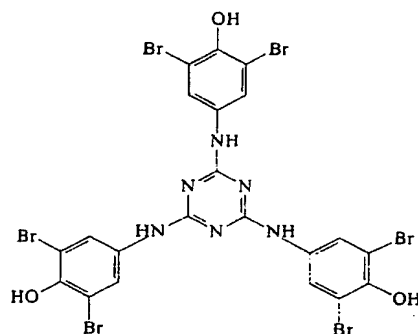
Each R is independently halo, hydroxy, mercapto, alkyl having 1-4 carbon atoms, alkoxy having 1-4 carbon atoms, thioalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, NR^1R^2 , nitro, cyano, CF_3 , COOH , SO_3H ,
 10 $\text{SO}_2\text{NR}^1\text{R}^2$, or SO_2F , where R^1 and R^2 are each independently hydrogen or a $\text{C}_1\text{-C}_4$ alkyl group.

Preferably, each R is independently hydroxy, alkoxy having 1 to 4 carbon atoms, fluoromethyl, or halogen. More preferably, each R is independently hydroxy or halo. Preferred halo is bromo or chloro. A particularly useful compound
 15 of the invention is 2,4,6-tri(3',5'-dichloro-4'-hydroxyphenyl)-amino-1,3,5-triazine.

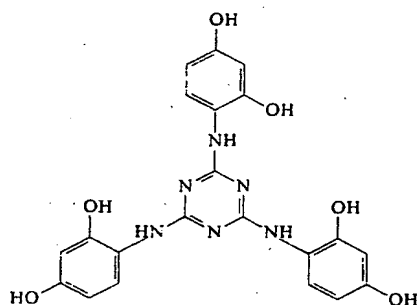
Preferred compounds include those where phenyl groups of formula I are substituted with one or more halogen or hydroxy groups. For example, useful compounds of the invention include those having the following structures:



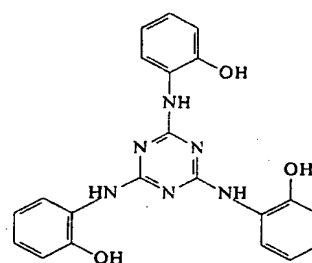
WHI-P199
2,4,6-bis(4'-hydroxyphenyl)amino-1,3,5-triazine



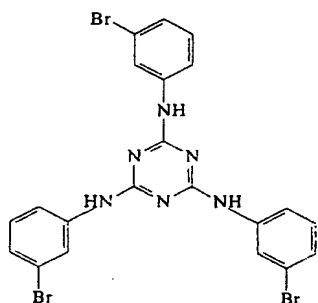
WHI-P200
2,4,6-tris(3',5'-dibromo-4'-hydroxyphenyl)amino-1,3,5-triazine



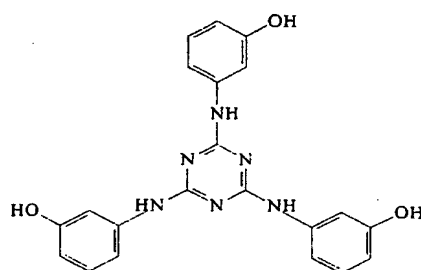
WHI-P203
2,4,6-tris(2',4'-dihydroxyphenyl)amino-1,3,5-triazine



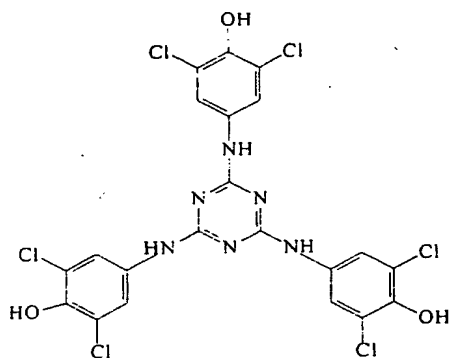
WHI-P205
2,4,6-tris(2'-hydroxyphenyl)amino-1,3,5-triazine



WHI-P206
2,4,6-tris(3'-bromophenyl)amino-1,3,5-triazine



WHI-P233
2,4,6-tris(3'-hydroxyphenyl)amino-1,3,5-triazine



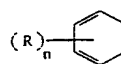
WHI-P239

2,4,6-Tri(3',5'-dichloro-4'-hydroxyphenyl)
amino-1,3,5-triazine

5

Although the triazine compounds of invention has been exemplified

above where each of the three substituted phenyl groups



are identical, it

is understood that each substituted phenyl group can be the same or different.

Synthesis of compounds where the substituted phenyl groups are identical is more

10 easily accomplished, however synthetic schemes for the production of compounds
having different substituted phenyl groups are known.

The compounds of the invention are capable of forming both
pharmaceutically acceptable acid addition and/or base salts. Base salts are formed
with metals or amines, such as alkali and alkaline earth metals or organic amines.

15 Examples of metals used as cations are sodium, potassium, magnesium, calcium,
and the like. Also included are heavy metal salts such as for example silver, zinc,
cobalt, and cerium. Examples of suitable amines are N,N'-dibenzylethylenediamine,
chloroprocaine, choline, diethanolamine, ethylenediamene, N-methylglucamine, and
procaine.

20 Pharmaceutically acceptable acid addition salts are formed with
organic and inorganic acids. Examples of suitable acids for salt formation are
hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic,
gluconic, fumaric, succinic, ascorbic, maleic, methanesulfonic, and the like. The
salts are prepared by contacting the free base form with a sufficient amount of the
25 desired acid to produce either a mono or di, etc. salt in the conventional manner.

The free base forms may be regenerated by treating the salt form with a base. For

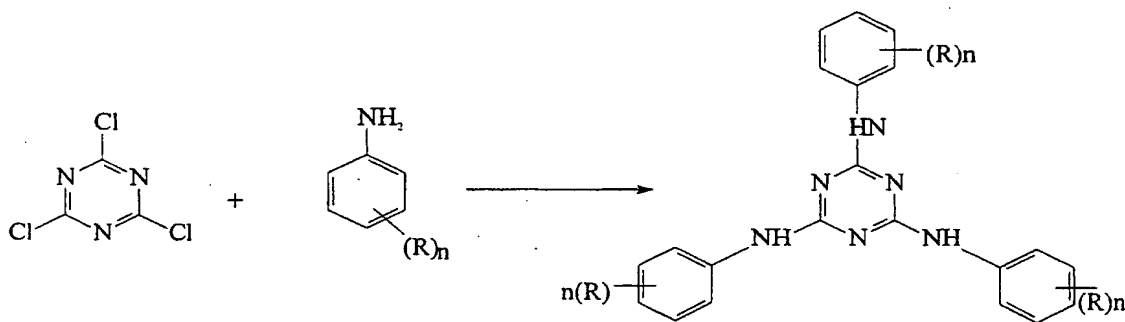
example, dilute solutions of aqueous base may be utilized. Dilute aqueous sodium hydroxide, potassium carbonate, ammonia, and sodium bicarbonate solutions are suitable for this purpose. The free base forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but the salts are otherwise equivalent to their respective free base forms for the purposes of the invention.

Melamine compounds of the present invention, such as the exemplary melamine derivative, 2,4,6-tri(3',5'-dichloro-4'-hydroxyphenyl)-amino-1,3,5-triazine (WHI-P239), can be prepared by the condensation of cyanuric chloride with 3, 5-dichloro-4-hydroxyaniline as shown in **Scheme 1**. This synthesis scheme serves to illustrate the general scheme that can be used for the synthesis of other melamine derivatives of the invention. Reactants for the synthesis of WHI-P239 and other melamine derivatives of the invention are commercially available and/or prepared by known methods.

Synthesis procedure of Melamine derivatives

To a solution of cyanuric chloride in THF is added the desired substituted aniline (1:3 molar ratio) with stirring for about 2-4 hours at room temperature. The reaction mixture is then refluxed 1-2 days and an excess amount of Et₃N is added. All solvent is evaporated and the crude is recrystallized from DMF to yield the desired melamine product. The synthetic procedure is diagramed below in Scheme 1.

Scheme 1



To prepare compounds of the invention where the three substituted phenyl groups are not identical, a mixture of anilines can be used in the reaction mixture. Alternatively, additional sites (e.g., Cl) can be blocked for selective addition of one substituted phenyl group at a time. Further, selective modification of the substituted phenyl groups may be performed after synthesis of the melamine derivative by known methods.

Cytotoxic Compounds

The compounds of the invention are effective cytotoxic agents, for example, against tumor cells such as leukemic and breast cancer cells. In the methods of the invention, the cytotoxic effects of melamine derivatives are achieved by contacting cells, such as tumor cells, with micromolar amounts of the inhibitory compound. By way of example, a particularly useful anti-tumor agent is 2,4,6-tri-(3',5'-dichloro-4'-hydroxyphenyl)-amino-1,3,5-triazine (P-239) as shown in the Examples below.

Tumor Treatment

The compounds of the invention can be used in methods of tumor treatment, for example, by administering to a subject a compound of the invention in order to achieve an inhibition of tumor cell growth, a killing of tumor cells, induced apoptosis, and/or increased patient survival time.

The cytotoxic compounds of the invention are suitable for use in mammals. As used herein, "mammals" means any class of higher vertebrates that nourish their young with milk secreted by mammary glands, including, for example, humans, rabbits, and monkeys.

Administration Methods

The compounds of the present invention can be formulated as pharmaceutical compositions and administered to a mammalian host, including a human patient, in a variety of forms adapted to the chosen route of administration. The compounds are preferably administered in combination with a pharmaceutically

acceptable carrier, and may be combined with or conjugated to specific delivery agents, including targeting antibodies and/or cytokines.

The compounds can be administered by known techniques, such as orally, parentally (including subcutaneous injection, intravenous, intramuscular, intrasternal or infusion techniques), by inhalation spray, topically, by absorption through a mucous membrane, or rectally, in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants or vehicles. Pharmaceutical compositions of the invention can be in the form of suspensions or tablets suitable for oral administration, nasal sprays, creams, sterile injectable preparations, such as sterile injectable aqueous or oleagenous suspensions or suppositories.

For oral administration as a suspension, the compositions can be prepared according to techniques well-known in the art of pharmaceutical formulation. The compositions can contain microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners or flavoring agents. As immediate release tablets, the compositions can contain microcrystalline cellulose, starch, magnesium stearate and lactose or other excipients, binders, extenders, disintegrants, diluents and lubricants known in the art.

For administration by inhalation or aerosol, the compositions can be prepared according to techniques well-known in the art of pharmaceutical formulation. The compositions can be prepared as solutions in saline, using benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons or other solubilizing or dispersing agents known in the art.

For administration as injectable solutions or suspensions, the compositions can be formulated according to techniques well-known in the art, using suitable dispersing or wetting and suspending agents, such as sterile oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid.

For rectal administration as suppositories, the compositions can be prepared by mixing with a suitable non-irritating excipient, such as cocoa butter,

synthetic glyceride esters or polyethylene glycols, which are solid at ambient temperatures, but liquefy or dissolve in the rectal cavity to release the drug.

Preferred administration routes include orally, parenterally, as well as intravenous, intramuscular or subcutaneous routes.

5 More preferably, the compounds of the present invention are administered parenterally, i.e., intravenously or intraperitoneally, by infusion or injection. In one embodiment of the invention, the compounds may be administered directly to a tumor by tumor injection; or by systemic delivery by intravenous injection.

10 Solutions or suspensions of the compounds can be prepared in water, isotonic saline (PBS) and optionally mixed with a nontoxic surfactant. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, DNA, vegetable oils, triacetin and mixtures thereof. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

15 The pharmaceutical dosage form suitable for injection or infusion use can include sterile, aqueous solutions or dispersions or sterile powders comprising an active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and
20 storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol such as glycerol, propylene glycol, or liquid polyethylene glycols and the like, vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size, in
25 the case of dispersion, or by the use of nontoxic surfactants. The prevention of the action of microorganisms can be accomplished by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be desirable to include isotonic agents, for example, sugars, buffers, or sodium chloride. Prolonged absorption of
30 the injectable compositions can be brought about by the inclusion in the composition

of agents delaying absorption--for example, aluminum monostearate hydrogels and gelatin.

Sterile injectable solutions are prepared by incorporating the compounds in the required amount in the appropriate solvent with various other ingredients as enumerated above and, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

10

Conjugation to a Targeting Moiety

The compound of the invention can be targeted for specific delivery to the cells to be treated by conjugation of the compounds to a targeting moiety. Targeting moiety useful for conjugation to the compounds of the invention include antibodies, cytokines, and receptor ligands expressed on the cells to be treated.

15

The term "conjugate" means a complex formed with two or more compounds.

The phrase "targeting moiety" means a compound which serves to deliver the compound of the invention to a specific site for the desired activity.

Targeting moieties include, for example, molecules which specifically bind molecules present on a cell surface. Such targeting moieties useful in the invention include anti-cell surface antigen antibodies. Cytokines, including interleukins, factors such as epidermal growth factor (EGF), and the like, are also specific targeting moieties known to bind cells expressing high levels of their receptors.

20

Particularly useful targeting moieties for targeting the compounds of the invention to cells for therapeutic activity include those ligands that bind antigens or receptors present on the tumor cells to be treated. For example, antigens present on B-lineage cancer cells, such as CD19, can be targeted with anti-CD19 antibodies such as B43. Antibody fragments, including single chain fragments, can also be used. IL4 can also be used to target B-cells. Cancer cells expressing EGF or IGF receptors can be targeted with the binding ligand. Other such ligand-receptor

25

30

binding pairs are known in the scientific literature for specific cancers. Methods for producing conjugates of the compounds of the invention and the targeting moieties are known.

5 Useful Dose

When used *in vivo* to kill tumor cells, the administered dose is that effective to have the desired effect, such as sufficient to reduce or eliminate tumors. Appropriate amounts can be determined by those skilled in the art, extrapolating using known methods and relationships, from the *in vitro* data provided in the

10 Examples.

In general, the dose of the novel melamine derivatives compounds effective to achieve tumor cell apoptosis, reduction in tumors, and increased survival time, is 1-100 mg/kg body weight/dose for a direct targeted administration.

The effective dose to be administered will vary with conditions
15 specific to each patient. In general, factors such as the disease burden, tumor location (exposed or remote), host age, metabolism, sickness, prior exposure to drugs, and the like contribute to the expected effectiveness of a drug. One skilled in the art will use standard procedures and patient analysis to calculate the appropriate dose, extrapolating from the data provided in the Examples.

20 In general, a dose which delivers about 1-100 mg/kg body weight is expected to be effective, although more or less may be useful.

In addition, the compositions of the invention may be administered in combination with other anti-tumor therapies. In such combination therapy, the administered dose of the melamine derivatives may be less than for single drug
25 therapy.

EXAMPLES

The invention may be further clarified by reference to the following Examples, which serve to exemplify some of the embodiments, and not to limit the
30 invention in any way.

Example 1

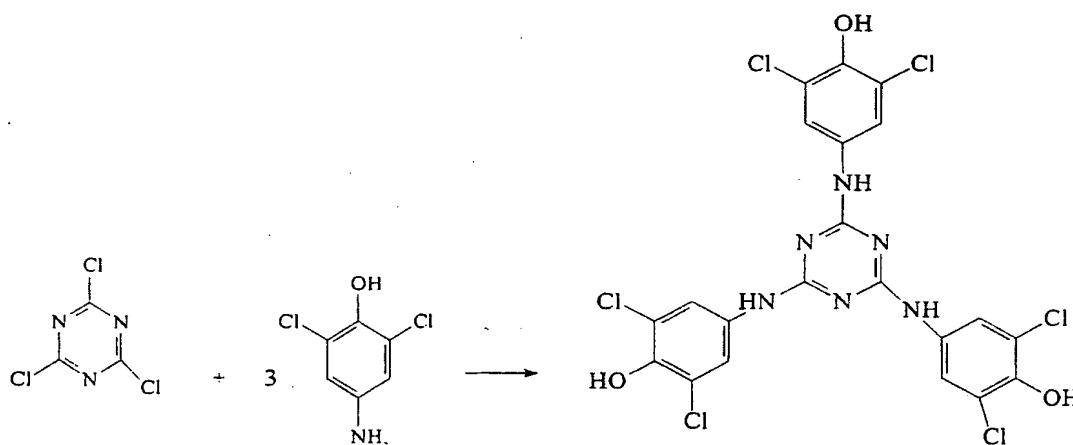
Synthesis and Characterization of

2,4,6-tri(3',5'-dichloro-4'-hydroxyphenyl)-amino-1,3,5-triazine(WHI-P239)

5 All chemicals were purchased from the Aldrich Chemical Company, Milwaukee, Wisconsin, and were used directly for synthesis. Anhydrous solvents such as acetonitrile, methanol, ethanol, ethyl acetate, tetrahydrofuran, chloroform, and methylene chloride were obtained from Aldrich as sure seal bottles under nitrogen and were transferred to reaction vessels by cannulation. All reactions were
10 carried out under a nitrogen atmosphere.

The compound 2,4,6-tri(3',5'-dichloro-4'-hydroxyphenyl)-amino-1,3,5-triazine(WHI-P239) was prepared through the condensation of cyanuric chloride with 3, 5-dichloro-4-hydroxylaniline as shown in **Scheme 1**.

15

Scheme 1

20

Specifically for the synthesis of WHI-P239, the following method was used. To a solution of 920 mg (5 mmol) of cyanuric chloride in 500 ml of THF was added 2.62 g (15 mmol) of 4-amino-2, 6-dichlorophenol with stirring for 2 hours at room temperature. The reaction mixture was then refluxed 15 hours and an excess amount of Et₃N was added. All solvent was evaporated and the crude was recrystallized from DMF to yield 2.4 g of product. Yield 82.53%. m.p. > 300.0 °C.

The following melamine compounds were synthesized as described above and characterized. Each structure is shown below in Table 1. The identifying analytical test results for each compound are also shown below. Proton and carbon Nuclear Magnetic Resonance (¹H and ¹³C NMR) spectra were recorded on a Mercury 2000 Varian spectrometer operating at 300 MHz and 75 MHz, respectively, using an automatic broad band probe. Unless otherwise noted, all NMR spectra were recorded in CDCl₃ at room temperature. ¹H chemical shifts are quoted in parts per million (δ in ppm) downfield from tetramethyl silane (TMS), which was used as an internal standard at 0 ppm and s, d, t, q, m designate singlet, doublet, triplet, quartet and multiplet, respectively. Melting points were determined using a Fisher-Johns melting apparatus and are uncorrected. UV spectra were recorded using a Beckmann Model # DU 7400 UV/V is spectrometer with a cell path length of 1 cm. Methanol was used as the solvent for the UV spectra. Fourier Transform Infrared spectra were recorded using an FT-Nicolet model Protege #460 instrument. The infrared spectra of the liquid samples were run as neat liquids using KBr discs. The KBr pellet method was used for all solid samples. The GC/mass spectrum analysis was conducted using a Hewlett-Packard GC/mass spectrometer model # 6890 equipped with a mass ion detector and Chem Station software. The temperature of the oven was steadily increased from 70°C to 250°C and the carrier gas was helium.

2,4,6-Tri(4'-hydroxyphenyl)-amino-1,3,5-triazine(WHI-P199).

Yield 82.53%, m.p. 280.0-282.0 °C. ¹H NMR(DMSO-d₆): d 10.16(s, brod, 3H, -NH), 9.47(s, 3H, -OH), 7.35(s, 6H, 2',6'-H), 6.76(s, 6H, 3',5' -H). UV(MeOH): 206.0, 276.0, 349.0 nm. IR(KBr)_umax : 3372, 3257, 2935, 1626, 1512, 1380, 1225 cm⁻¹. GC/MS m/z 403(M⁺+1, 25.45) , 402(M⁺,100.00) , 401(M⁺-1, 22.20), 311(22.20) , 310(12.91), 309(17.59.), 135(15.56), 134(19.26).

2,4,6-Tri(3',5'-dibromo-4'-hydroxyphenyl)-amino-1,3,5-triazine(WHI-P200).

Yield 91.63%, m.p. 248.0-250.0 °C. ¹H NMR(DMSO-d₆): d 9.36(s, , 3H, -NH), 7.84(s, 6H, 2', 6'-H). UV(MeOH): 203.0, 214.0 , 280.0 nm. IR(KBr)_umax : 3473, 3381, 3070, 2993, 1624, 1500, 1471, 1161 cm⁻¹. GC/MS m/z 882(M⁺+6, 7.51) , 881(M⁺+5,30.36), 880(M⁺+4,18.95) , 879(M⁺+3, 74.63), 878(M⁺+2, 25.72), 877(M⁺+1, 100.00), 876(M⁺,100.00) , 875(M⁺-1,76.04), 795(29.20), 614 (18.00), 613 (41.50).

2,4,6-Tri(2',4'-Dihydroxyphenyl)-amino-1,3,5-triazine(WHI-P203)

Yield 88.61%, m.p.205.0 °C(dec.). ¹H NMR(DMSO-d₆): d 10.60(s, 3H, -NH), 9.70(s, 6H, -OH), 7.12(d, J_{5'}, 6'=8.7 Hz, 3H, 6'-H), 6.55(d, J_{5'}, 3'=2.7 Hz, 3H, 3'-H), 7.12(dd, J_{5'}, 6'=8.7 Hz, J_{5'}, 3'=2.7 Hz, 3H, 5'-H). ¹³C NMR(DMSO-d₆): d 158.12, 151.7, 124.60, 110.06, 106.30, 103.22. UV(MeOH):. 207.0, 265.0, 296.0 nm. IR _umax (KBr) : 3211, 2868, 1626, 1510, 1457, 1346, 1038 cm⁻¹.

2,4,6-Tri(2'-hydroxyphenyl)-amino-1,3,5-triazine(WHI-P205)

Yield 98.00%; m.p. 225.0-227.0 °C. ¹H NMR(DMSO-d₆): d 10.02(s, broad, 3H, -NH), 9.47(s, 3H, -OH), 7.58-6.82(m, 12H, Ph-H). UV(MeOH):. 207.0, 265.0, 296.0 nm. IR(KBr)_umax: 3211, 2868, 1626, 1510, 1457, 1346, 1038 cm⁻¹.

2,4,6-Tri(3'-bromophenyl)-amino-1,3,5-triazine(WHI-P206).

Yield 82.53%, m.p.193.0-196.0 °C. ¹H NMR(DMSO-d₆) : d 9.31(s, brod, 3H, -NH), 7.75-6.99(m, 12H, 2', 4', 5', 6' -H). UV(MeOH): 217.0, 276.0, 328.0 nm. IR(KBr)_umax: 3398, 3272, 2929, 1630, 1567, 1478, 1220, 1066 cm⁻¹. GC/MS m/z 592(M⁺+1,99.47), 591(M⁺, 55.06), 590(M⁺-1, 100,00), 511(5.21) , 431(3.49), 378(19.79).

2,4,6-Tri(3'-hydroxyphenyl)-amino-1,3,5-triazine(WHI-P233).

Yield 98.00%, m.p.270.0-271.0 °C. ¹H NMR(DMSO-d₆): d 11.28(s, 3H, -NH), 9.91(s, 3H, -OH), 7.27-6.52 (m, 12H, 2',4',5',6'-H). UV(MeOH):. 219.0, 272.0, 288.0 nm. IR(KBr)_umax: 3325, 3113, 2877, 1608, 1456, 1363, 1159 cm⁻¹. Found: C, 57.44; H, 4.41; N, 19.12. C₂₁H₁₈N₆O₃.HCl requires: C, 57.44; H, 4.41; N, 19.12%.

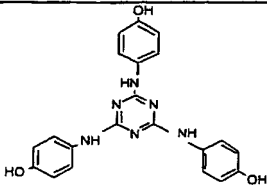
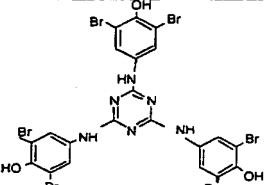
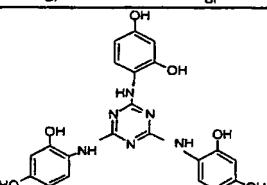
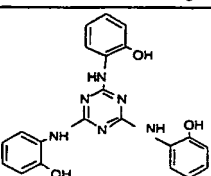
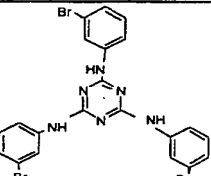
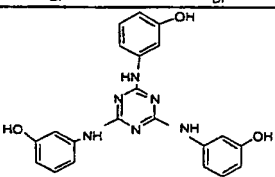
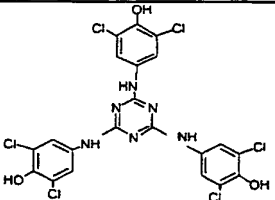
2,4,6-Tri(3',5'-dichloro-4'-hydroxyphenyl)-amino-1,3,5-triazine(WHI-P239).

Yield 79.21%; m.p. > 300.0 °C. ¹H NMR(DMSO-d₆): d 9.44(s, 3H, -HN), 7.69(s, 6H, Ph-H). UV(MeOH): 211.0, 249.0, 339.0 nm. IR(KBr)_{max}: 3241, 2839, 2783, 1635, 1580, 1514, 1420, 1360, 1281 cm⁻¹.

5

The following melamine derivatives described in Table 1 were prepared according to the above method.

Table 1. Melamine Derivatives of the Invention

No	Name	Structure	Formula	MW
1	P-199		$C_{21}H_{18}N_6O_3$	402
2	P-200		$C_{21}H_{12}Br_6N_6O_3$	877
3	P-203		$C_{21}H_{18}N_6O_6$	450
4	P-205		$C_{21}H_{18}N_6O_3$	402
5	P-206		$C_{18}H_{15}Br_3N_6$	591
6	P-233		$C_{21}H_{18}N_6O_3$	402
7	P-239		$C_{21}H_{12}Cl_6N_6O_3$	606

Example 2**Cytotoxicity of Melamine Derivatives**

The cytotoxicity of melamine derivatives against tumor cells was
5 evaluated in leukemic cells and breast cancer cells.

Cytotoxicity Assay

Cytotoxicity of various compounds against tumor cells was
performed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium
10 bromide) assay (Boehringer Mannheim Corp., Indianapolis, IN). Unless otherwise
specified, all cell lines were obtained from the American Type Culture Collection
(ATCC). Briefly, exponentially growing cells were seeded into a 96-well plate at a
density of 2.5×10^4 cells/well and incubated for 36 hours at 37°C prior to drug
exposure. On the day of treatment, culture medium was carefully aspirated from
15 the wells and replaced with fresh medium containing WHI-P239 at concentrations
ranging from 0.1 to 250 μ M. Triplicate wells were used for each treatment.

Human leukemic cell lines (NALM-6, MOLT-3), human breast
tumor cells (BT20), prostate cancer cells (PC3), and glioblastoma cells (U373)
were obtained from the American Type Culture Collection and maintained as a
20 continuous cell line in Dulbecco's modified Eagles's medium supplemented with
10% fetal bovine serum and antibiotics.

The cells were incubated with the various compounds for 24-36 hours
at 37°C in a humidified 5% CO₂ atmosphere. To each well, 10 μ l of MTT (0.5
mg/ml final concentration) was added and the plates were incubated at 37°C for 4
25 hours to allow MTT to form formazan crystals by reacting with metabolically active
cells. The formazan crystals were solubilized overnight at 37°C in a solution
containing 10% SDS in 0.01 M HCl. The absorbence of each well was measured in
a microplate reader (Labsystems) at 540 nm and a reference wavelength of 690 nm.
To translate the OD₅₄₀ values into the number of live cells in each well, the OD₅₄₀
30 values were compared to those on standard OD₅₄₀ - versus - cell number curves
generated for each cell line. The percent survival was calculated using the formula

shown below. The IC_{50} values were calculated by non-linear regression analysis and are shown in Table 2.

$$\% \text{ Survival} = \frac{\text{live cell number [test]}}{\text{live cell number [control]}} \times 100$$

5

Table 2: Cytotoxicity in Tumor Cells

Compound	IC_{50} (μM)			
	NALM-6 Leukemia	BT-20 Breast Cancer	PC-3 Prostate Cancer	U373 Brain Tumor
WHI-P199	>250	240.2	221.3	>250
WHI-P233	5.1	66.1	45.1	104.4
WHI-P205	21.5	107.6	102.1	224.3
WHI-P200	10.1	27.1	64.5	>250
WHI-P239	9.8	13.2	31.5	155.1
WHI-P206	>250	>250	>250	>250

The most effective compound against leukemia cells was WHI-P233. The
 10 ranking order of the antileukemic potency was: WHI-P233 ($IC_{50} = 5.1 \mu\text{M}$) > WHI-P239 ($IC_{50} = 9.8 \mu\text{M}$) > WHI-P200 ($IC_{50} = 10.1 \mu\text{M}$) > WHI-P205 ($IC_{50} = 21.5 \mu\text{M}$). Compounds WHI-P199 and WHI-P206 had no antileukemic activity ($IC_{50} > 250 \mu\text{M}$).

The most effective compound against breast cancer cells was WHI-P239. The ranking order of the anti-breast cancer potency was: WHI P239 ($IC_{50} =$
 15 $13.2 \mu\text{M}$) > WHI-P200 ($IC_{50} = 27.1 \mu\text{M}$) > WHI-P233 ($IC_{50} = 66.1 \mu\text{M}$) > WHI-P205 ($IC_{50} = 107.6 \mu\text{M}$) > WHI-P199 ($IC_{50} = 240.2 \mu\text{M}$). Compound WHI-p206 exhibited no cytotoxic activity against breast cancer cells ($IC_{50} > 250 \mu\text{M}$).

The most effective compound against prostate cancer cells was WHI-P239. The ranking order of anti-prostate cancer activity was: WHI-P239 ($IC_{50} = 31.5 \mu\text{M}$)
 20 > WHI-P233 ($IC_{50} = 45.1 \mu\text{M}$) > WHI-P200 ($IC_{50} = 64.5 \mu\text{M}$) > WHI-P205 ($IC_{50} =$

102.1 μM) > WHI-P199 (IC_{50} = 221.3 μM) > WHI-P206 (IC_{50} > 250 μM). These compounds were least active against brain tumor cells.

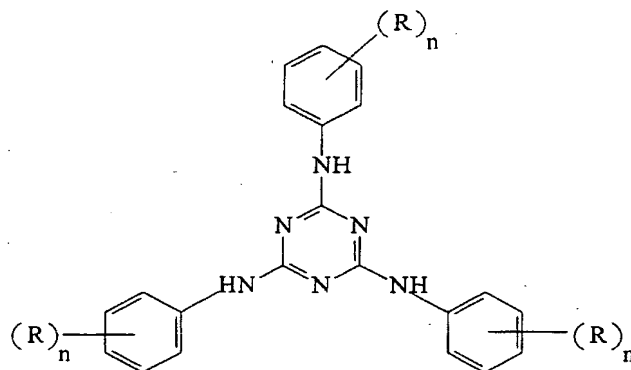
The ranking order of anti-glioblastoma activity was: WHI-P233 (IC_{50} = 104.4 μM) > WHI-P239 (IC_{50} = 155.1 μM) > WHI-P205 (IC_{50} = 224.3 μM).

- 5 Compounds WHI-P200 and WHI-P206 exhibited no cytotoxic activity against brain tumor cells.

All publications, patents, and patent documents described herein are incorporated by reference as if fully set forth. The invention described herein may
10 be modified to include alternative embodiments. All such obvious alternatives are within the spirit and scope of the invention, as claimed below.

We claim:

1. A compound of the formula



wherein each n is independently an integer of 1 to 5;

each R is independently halogen, hydroxy, mercapto, acyl, thioalkyl, (C₁-C₄) alkyl, (C₁-C₄) alkoxy, (C₁-C₄) alkylthio, (C₁-C₄) hydroxyalkyl, NR¹R², nitro, cyano, CF₃, COOH, SO₃H, SO₂NR¹R² or SO₂F, where R¹ and R² are each independently hydrogen or (C₁-C₄) alkyl; or

a pharmaceutically acceptable acid addition salt thereof.

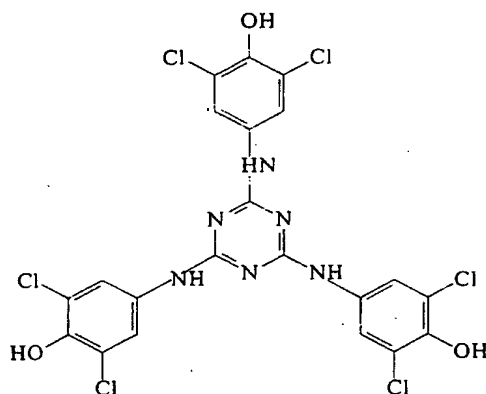
2. The compound of claim 1, wherein each R is independently hydroxy, (C₁-C₄) alkoxy, trifluoromethyl, or halo.

3. The compound of claim 2, wherein each R is independently, hydroxy or halo.

4. The compound of claim 2, wherein halo is bromo or chloro.

22

5. A compound of the formula



6. The compound of claim 1, wherein at least one R is halo.
7. The compound of claim 1, wherein at least one R is hydroxy.
8. The compound of claim 6, wherein at least one R is bromo or chloro.
9. The compound of claim 1, wherein R is 4'-hydroxy.
10. The compound of claim 1, wherein R is 2',4'-dihydroxy.
11. The compound of claim 1, wherein R is 3'-bromo.
12. The compound of claim 1, wherein R is 3',5'-dibromo-4'-hydroxy.
13. The compound of claim 1, wherein R is 2'-hydroxy.
14. The compound of claim 1, wherein R is 3'-hydroxy.
15. The compound of claim 1, wherein R is 3',5'-dichloro-4'-hydroxy.

16. A pharmaceutical composition comprising a therapeutically effective amount of the compound of claim 1 and a pharmaceutically acceptable carrier or diluent.

5 17. A method for inhibiting growth of tumor cells in a subject comprising administering to said subject a compound of claim 1.

18. The method of claim 17, wherein said inhibiting comprises inducing apoptosis of said tumor cells.

10

19. A method of treating cancer in a subject comprising administering a therapeutically effective amount of the compound of claim 1.

20. The method of claim 19, wherein said cancer is leukemia.

15

21. The method of claim 19, wherein said cancer is breast cancer.

22. A method for inducing cytotoxicity in a cell comprising:
administering to said cell a cytotoxic dose of the compound of claim

20 1.

23. The method of claim 22, wherein said cell is a cancer cell.

24. The method of claim 22, wherein said compound is

25

2,4,6-tri(4'-hydroxyphenyl)amino-1,3,5-triazine (WHI-P199);

2,4,6-tri (3',5'-dibromo-4'-hydroxy phenyl) amino-1,3,5-triazine (WHI-P200);

2,4,6-tri(2',4'-dihydroxy phenyl)amino-1,3,5-triazine (WHI-P203);

2,4,6-tri(2'-hydroxy phenyl)amino-1,3,5-triazine (WHI-P205);

2,4,6-tri(3'-bromo phenyl)amino-1,3,5-triazine (WHI-P206);

30

2,4,6-tri(3'-hydroxy phenyl)amino-1,3,5-triazine (WHI-P233); or

2,4,6-tri(3',5'-dichloro-4'-hydroxylphenyl) amino-1,3,5-triazine (WHI-P239).

25. The method of claim 24, wherein said compound is
2,4,6-tri(3',5'-dichloro-4'-hydroxyphenyl)-amino-1,3,5-triazine (WHI-P239).

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/05319

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D251/70 A61K31/53 A61P35/00 A61P35/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 06781 A (INSTITUTE OF CANCER RESEARCH ET AL.) 31 March 1994 (1994-03-31) page 1 -page 3 ---	1-25
A	WO 93 20056 A (JARMAN MICHAEL ET AL.) 14 October 1993 (1993-10-14) page 1 -page 3 ---	1-25
A	WO 93 10116 A (PFIZER INC.) 27 May 1993 (1993-05-27) page 1 -page 8, line 33 ---	1-25
A	EP 0 796 851 A (3V SIGMA S.P.A) 24 September 1997 (1997-09-24) page 2 ---	1
	-/--	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

4 July 2000

Date of mailing of the international search report

12/07/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Kyriakakou, G

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/05319

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 517 104 A (SIGMA PRODOTTI CHIMICI S. P.A) 9 December 1992 (1992-12-09) page 2 ---	1
A	US 5 854 244 A (MICHAEL JARMAN ET AL.) 29 December 1998 (1998-12-29) the whole document ---	1-25
A	US 5 536 722 A (JOTHAM W. COE ET AL.) 16 July 1996 (1996-07-16) the whole document ---	1-25
A	US 5 534 625 A (MICHAEL JARMAN ET AL.) 9 July 1996 (1996-07-09) the whole document -----	1-25

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 00/05319

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9406781	A	31-03-1994	AU	4825993 A	12-04-1994
WO 9320056	A	14-10-1993	AT	168105 T	15-07-1998
			AU	676677 B	20-03-1997
			AU	3894293 A	08-11-1993
			DE	69319590 D	13-08-1998
			DE	69319590 T	12-11-1998
			EP	0632805 A	11-01-1995
			ES	2118945 T	01-10-1998
			JP	7505380 T	15-06-1995
			US	5534625 A	09-07-1996
			US	5854244 A	29-12-1998
WO 9310116	A	27-05-1993	AU	2599992 A	15-06-1993
			EP	0613477 A	07-09-1994
			FI	942188 A	11-05-1994
			HU	69722 A	28-09-1995
			JP	7506084 T	06-07-1995
			MX	9206468 A	01-05-1993
			NO	941785 A	11-05-1994
			PT	101046 A	28-02-1994
			US	5536722 A	16-07-1996
			ZA	9208683 A	11-05-1994
EP 796851	A	24-09-1997	IT	MI960576 A	22-09-1997
			US	5801244 A	01-09-1998
EP 517104	A	09-12-1992	IT	1247973 B	05-01-1995
			AT	145643 T	15-12-1996
			DE	69215434 D	09-01-1997
			DE	69215434 T	27-03-1997
			DK	517104 T	05-05-1997
			US	5393517 A	28-02-1995
			US	5233040 A	03-08-1993
US 5854244	A	29-12-1998	AT	168105 T	15-07-1998
			AU	676677 B	20-03-1997
			AU	3894293 A	08-11-1993
			DE	69319590 D	13-08-1998
			DE	69319590 T	12-11-1998
			EP	0632805 A	11-01-1995
			ES	2118945 T	01-10-1998
			WO	9320056 A	14-10-1993
			JP	7505380 T	15-06-1995
			US	5534625 A	09-07-1996
US 5536722	A	16-07-1996	AU	2599992 A	15-06-1993
			EP	0613477 A	07-09-1994
			FI	942188 A	11-05-1994
			HU	69722 A	28-09-1995
			JP	7506084 T	06-07-1995
			MX	9206468 A	01-05-1993
			NO	941785 A	11-05-1994
			PT	101046 A	28-02-1994
			WO	9310116 A	27-05-1993
			ZA	9208683 A	11-05-1994
US 5534625	A	09-07-1996	AT	168105 T	15-07-1998

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 00/05319

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5534625 A		AU 676677 B	20-03-1997
		AU 3894293 A	08-11-1993
		DE 69319590 D	13-08-1998
		DE 69319590 T	12-11-1998
		EP 0632805 A	11-01-1995
		ES 2118945 T	01-10-1998
		WO 9320056 A	14-10-1993
		JP 7505380 T	15-06-1995
		US 5854244 A	29-12-1998

